BIOLOG

OmniLog® Phenotype MicroArray™ Software Release 1.2

User Guide

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OmniLog Phenotype MicroArray Software Release 1.2 User Guide

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1. Introducing OmniLog PM Software

In this section:
- **➔ OmniLog PM's Suite of Programs**
- **➔ OmniLog Software Steps**

The OmniLog® Phenotype MicroArray (PM) system and software include everything you need to incubate microbes. OmniLog PM is a dedicated system for phenotype microarray testing and includes the following components:

- Dedicated Windows®-based computer with preinstalled OmniLog PM software
- OmniLog Incubator/Reader

OMNILOG PM SYSTEM

OmniLog PM's Suite of Programs

OmniLog PM Software contains a suite of four programs that work in conjunction with the OmniLog reader and Biolog's PM MicroPlates. These programs allow you to generate kinetic PM MicroPlate data from the OmniLog reader, manage and analyze the data, export it in a variety of raw and processed forms, and generate reports.

Program 1: Data Collection

- Drives the OmniLog reader
- Allows you to load, read, and unload MicroPlates from the reader
- Creates a kinetic data file for each MicroPlate
- Creates files for use in the other three programs

Program 2: Data Management

- Allows you to view and edit individual MicroPlate data files

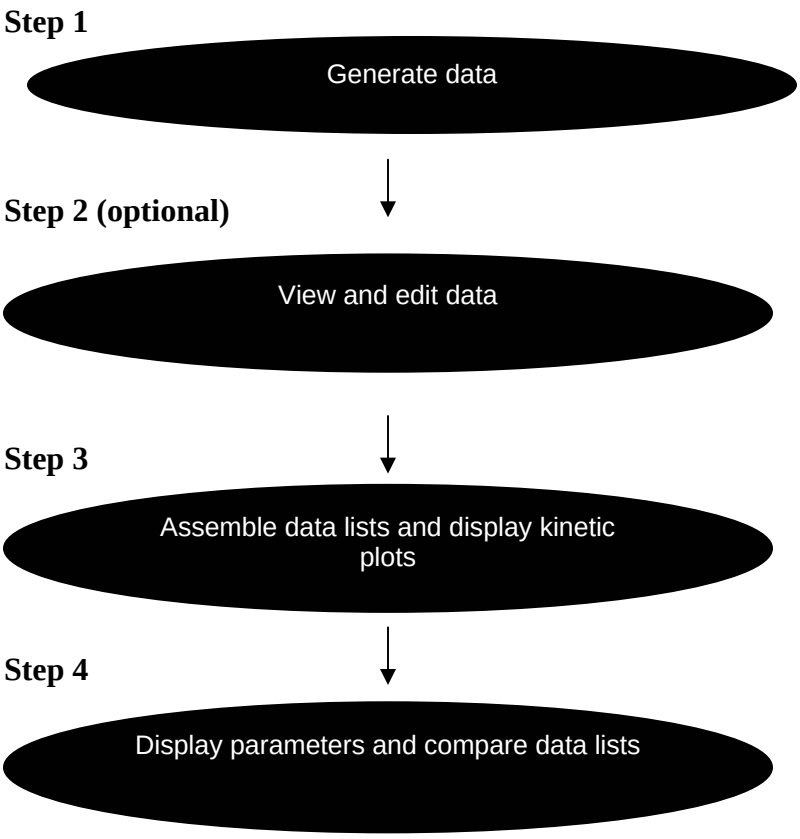
Program 3: File Management/Kinetic Analysis

- Allows you to assemble MicroPlate data files into data lists
- Displays kinetic plots of the data

Program 4: Parametric Analysis

- Takes data lists from the File Management/Kinetic Analysis program and calculates parameters from the kinetic data
- Allows you to compare two data lists

OmniLog PM Software Steps



Step 1: Generate data

The Data Collection program operates the OmniLog reader via the dedicated OmniLog computer. After you have inoculated a PM panel, load the MicroPlates into the OmniLog to begin data collection. The software guides you through the MicroPlate loading process, reads MicroPlates every 15 minutes during a user-defined incubation period, and walks you through the unload process.

Step 2: View and Edit Data

The Data Management program lets you view and edit MicroPlate information fields in the kinetic data files on a plate-by-plate basis. This allows you to correct any errors entered in the MicroPlate information fields during loading in the Data Collection program. This part of the software also lets you view the kinetic data for an individual MicroPlate and offers data visualization and reports tools for specialty MicroPlates and reports. You can skip this step unless you need to edit MicroPlate information fields or view a special individual MicroPlate.

Step 3: Make data lists and kinetic displays

The File Management/Kinetic portion of the software lets you assemble several MicroPlates of data into a unified set called a “data list.” Data lists are the data file organizational format for the OmniLog PM software. These lists contains information about all the PM MicroPlates applicable to a given organism type or compound treatment and can handle replicate data runs of the same MicroPlate type. Once a data list is defined, you can view all the data on that list. OmniLog values in a data list are exportable to an Excel spreadsheet.

Step 4: View parametric data and generate reports

The Parametric program lets you take a data list, as defined by the File Management/Kinetic Analysis program, and generate an assortment of parameters. The program also allows you to compare parameters between two data lists. These parameters can then be displayed in various formats, output in reports, or exported into external files for further analysis.

2. Defining Data Lists and Displaying Kinetic Plots

In this section:
- ➔ **Converting File Format**
- ➔ **Populating a Worksheet List**
- ➔ **Assembling a Data List**
- ➔ **Displaying Kinetic Plots**
- ➔ **Averaging Data**
- ➔ **Exporting Data**

The File Management/Kinetic Plot module of the OmniLog PM software lets you assemble several MicroPlates of data into a single data list. Using this assembled data, the program displays kinetic plots for every well of a MicroPlate. The program can superimpose replicate plots, thereby allowing you to observe the reproducibility of your data. The data list also serves as the organizational format for the Parametric program.

The data file search feature allows you to select data files according to specific criteria such as organism, strain, gene/compound, plate type, temperature, project code, etc. This offers a comprehensive, streamlined search, including the ability to evaluate data collected over long periods of time.

DEFINITIONS

Data File
- ⇒ A single file containing all information and data for one MicroPlate
- ⇒ Contains plate number, inoculum organism, inoculum compound, etc.

Worksheet List:
- ⇒ A worksheet list contains all the files that meet the criteria of your data file search
- ⇒ Each row is a data file for one MicroPlate
- ⇒ Each column corresponds to MicroPlate type, gene/compound, organism, strain, and file name, with an 'other' column for comments

Data List:
- ⇒ A way of organizing data files by relatedness or by membership in a single experiment
- ⇒ Data files from any subset of 40 possible MicroPlates
- ⇒ A data list includes averaged data over multiple replicates of a single MicroPlate
- ⇒ Each row represents one MicroPlate type
- ⇒ Each additional column shows an additional replicate for a particular MicroPlate type
- ⇒ Data files in the same row are assumed to be replicates; their data is averaged for reports

Converting File Format

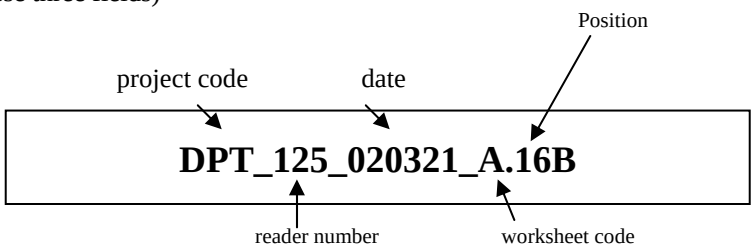
Data files may be named according to either an old or a new naming format. In order to get the maximum use of the OmniLog PM software, all files should be named according to new format, which has the extension 'oka.' For example, the data file search feature of the software only works on files named according to the new format.

FILE NAMING CONVENTIONS

Old data files

⇒ Four fields separated by underscores
⇒ Fields are: project code, reader number, date, worksheet code
⇒ File extension: [01-25][A,B] (these correspond to the position of the MicroPlate inside the OmniLog reader)
⇒ Reader number, date and position uniquely identify each MicroPlate (no two MicroPlates have identical values for these three fields)

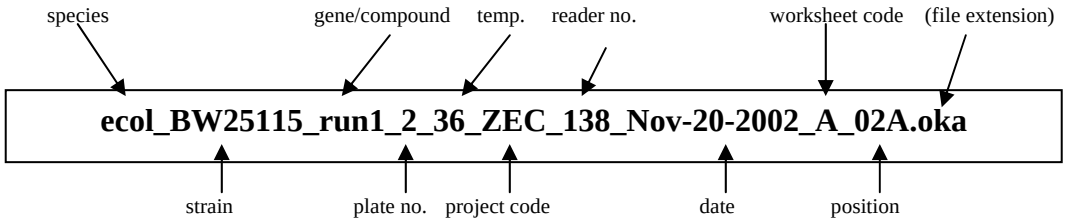
example:



New data files

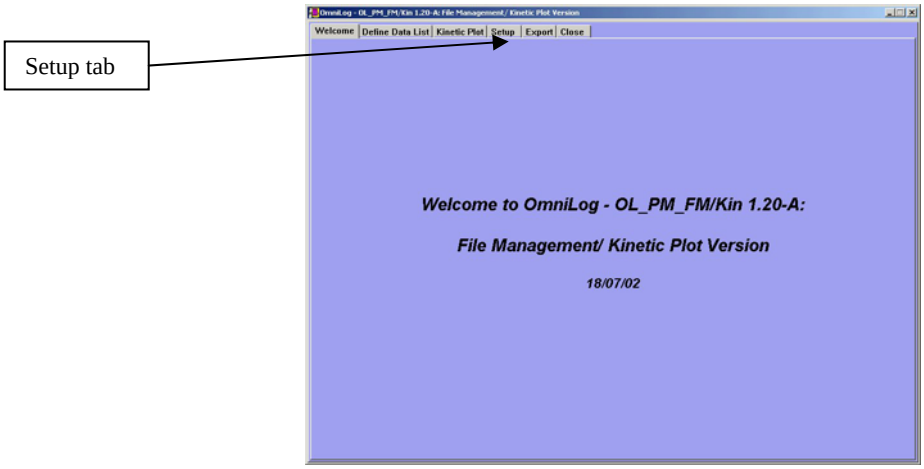
⇒ Eleven fields separated by underscores
⇒ Fields are: species, strain, gene/compound, other, MicroPlate number, temperature, project code, reader number, date, worksheet code, position
⇒ File extension: .oka (OmniLog kinetic version A)

example:



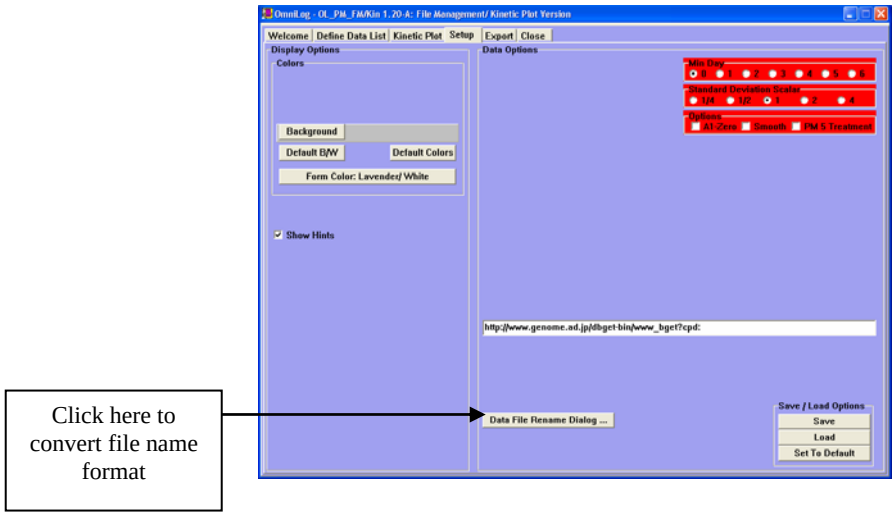
Converting file name format:

Once you launch the File Management/Kinetic Analysis program, a **Welcome** page appears, displaying name, version, and date information about the program.



WELCOME PAGE

1. Click the **Setup** tab on the menu bar.
2. Click the **Data File Rename Dialog** button at the bottom middle of the page.



3. The pop-up dialog displays two Windows file directory trees: the Origin directory (on the left) and the Destination directory (on the right). The Origin directory is the one that contains the data files to be copied and renamed (though the data file names will not be listed on the page). The Destination directory is the one in which you want the copies to ultimately reside. Most often, you will want the Origin and Destination directories to be the same (the software will do this by

default), but you do have the option of saving the renamed data files to a new directory. Navigate through the directories by double-clicking until you find the desired Origin directory.

Note: You may want to keep Windows Explorer open at the same time, so you can view the individual data files in a given folder and make sure you are selecting the desired folder.

4. Click the **Copy/Rename Data Files** button. The software will automatically rename the data files, assigning each one an 11-field file name. The old data files are not overridden; the software simply creates a new set of data files with new names. All the information in the old file name is contained in the new file name (with additional helpful identifiers at the front end of the name).

Note: You can use Windows Explorer to verify the new file names in the Destination directory folder. It's easy to distinguish between the old and new naming formats; the old file names are brief, while the new ones are longer.

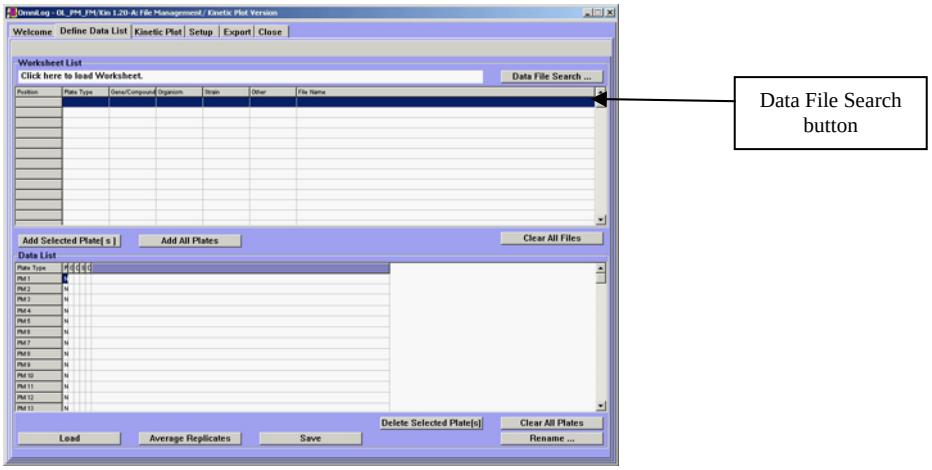
Populating a Worksheet List

Worksheet lists serve two functions:

- to list data files found during a new search
- to retrieve worksheet files created in the Data Collection program and to display them

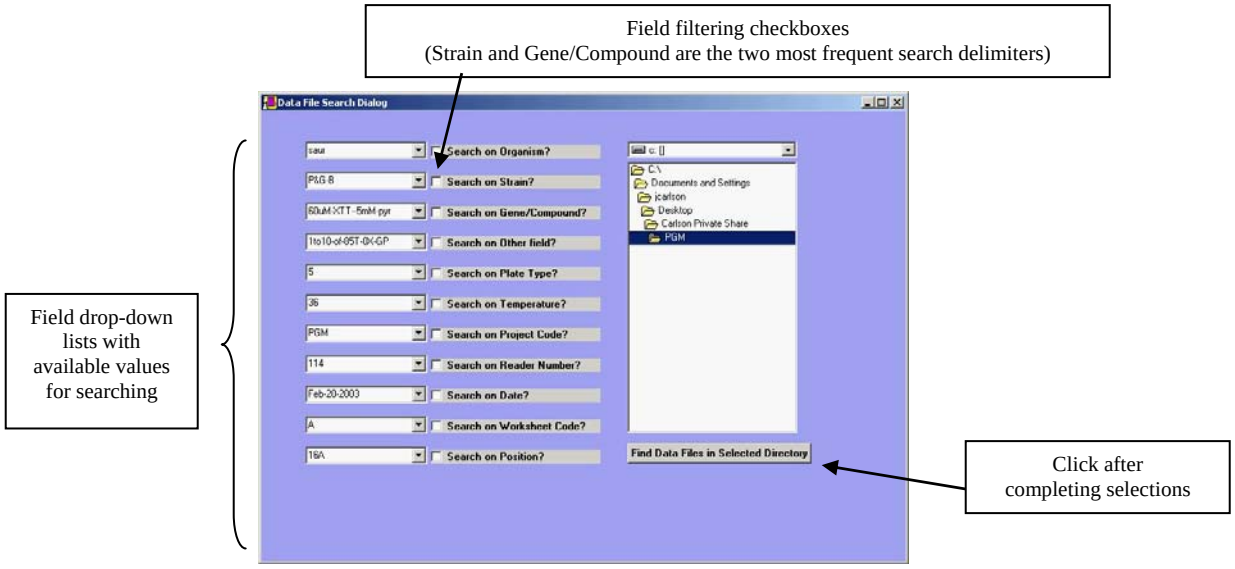
Conducting a new search

1. Click on the **Define Data List** tab on the menu bar.



DEFINE DATA LIST PAGE

2. To search for data files using specific criteria, click on the **Data File Search** button at the far right corner of the page. The **Data File Search Dialog** pops up.


Field filtering checkboxes
(Strain and Gene/Compound are the two most frequent search delimiters)
Field drop-down lists with available values for searching
Click after completing selections
Data File Search Dialog
Search on Organism?
Search on Strain?
Search on Gene/Compound?
Search on Other field?
Search on Plate Type?
Search on Temperature?
Search on Project Code?
Search on Reader Number?
Search on Date?
Search on Worksheet Code?
Search on Position?
Find Data Files in Selected Directory

DATA FILE SEARCH DIALOG

3. Use the directory tree on the right to locate the directory containing data files. The window will momentarily flash white as the computer scans the directory for files with the 'oka' extension. The drop-down lists on the left will be populated with all the values found for the 11 fields (these are the same fields used for re-naming the data files.)
4. Check the desired checkboxes to select the fields you want to filter the data files. You can check as many checkboxes as desired; this will narrow the search to only those data files that have matching values for all the selected fields. If you don't check any check boxes, the software will find all data files in the directory you selected.

Consecutive searches are cumulative in the worksheet list.

Note: If you narrow the search too much, you may get a message saying that no files were found; simply remove some of the checkmarks or select a different value for a field and the search may broaden.

5. For each of the fields you've checked, select a value from the corresponding drop-down list on the left. Click on the downward arrows to make each list drop down.
   - You can only select one value from a given drop-down list
   - Searches are cumulative; you can search on one value, then another, thereby locating files that match either value.
   - The results of subsequent searches are added to the bottom of the worksheet list.

If subsequent searches find a file that is already on the worksheet list, the program will give you a warning message. Ignore the message if you want to continue searching.

6. After you've made the desired selections, click the **Find Data Files in Selected Directory** button. The window will momentarily turn white while the data files are added to the worksheet list. Do not click anything until the original window color returns. Once searching is complete, you'll see the data files listed on the worksheet list.
7. Conduct as many searches as you like, selecting different fields and/or drop-down values as desired.

*Note: You do not need to close and re-open the **Data File Search Dialog** to perform subsequent searches.*

8. When you are satisfied with your search results as listed in the worksheet list, close the **Data File Search Dialog**. You'll return to the **Define Data List** page with the data files found according to the criteria you selected, in the order you conducted the searches.

Retrieving an existing worksheet list

1. To view a worksheet list you've previously created and saved, click in the box containing the words **Click here to load worksheet** at the top of the **Define Data List** page.
2. A Windows **Open File Dialog** will pop up; select the desired worksheet, then click **Open**.
3. The data files will populate the worksheet list. From here, you can use them to assemble a data list as you would files from a new search.

Assembling a Data List

Populating a worksheet list is an interim step in the process of compiling a data list. Data lists organize the data files for subsequent analysis in the Parametric program. Data lists allow you to:

- save a search
- display kinetic plots
- export files to external programs (such as Excel®)
- use the OmniLog parametric program

A data list is OmniLog's format for organizing data files. Each data list represents an experiment that consists of a particular group of compounds or strains in one set of media, with a certain number of replicates.

1. If you are satisfied with the data files in the worksheet list on the upper half of the **Define Data List** page, simply click the **Add All Plates** button. The software will copy all the listed data files into the data list on the lower half of the page.

Warning: If you click **Add Plates** multiple times, the software will duplicate the data files in the data list however many times you click.

- The first few (very narrow) columns at the left of the data list contain identifying information. You can expand these columns by clicking on the edge of the colored header at the top of the data list and dragging the column to the right. However, the information in these columns is already displayed in the 11-field file name column. Consequently, you'll most likely only need to expand these columns to view data files named according to the old format.

WHAT'S ON A DATA LIST?

Left Column:

⇒ Plate Type (unique identifier assigned to each type of PM MicroPlate, with a defined set of chemicals in all 96 wells)

⇒ This column lists a subset of the 40 possible PM MicroPlates types, depending on the MicroPlates used in that experiment

Expanding Columns:

⇒ These five expandable columns list the most common search criteria for the data files

⇒ To view fully, click on the edge of the header and drag the column to the right

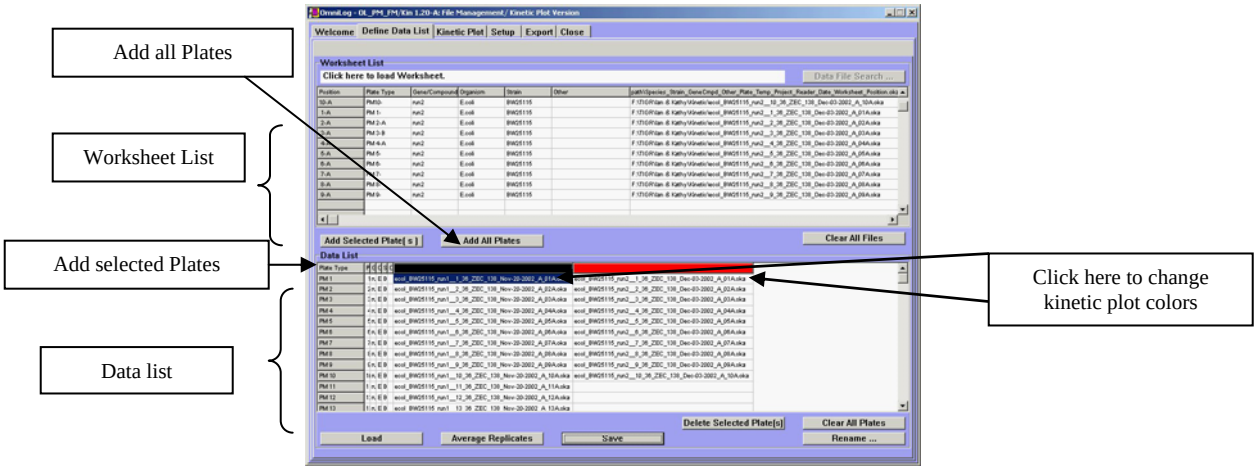
Data File Columns:

⇒ These wide column list the 11-field names of the data files found in your search

⇒ Color of header row matches color of kinetic plot corresponding to that column of data (color can be changed)

⇒ Each column corresponds to an additional replicate for each row that has an additional replicate. Note that a particular row need not have the same number of replicates.

⇒ You can add 119 replicate plates (when adding a replicate, the program adds a column to the right of the applicable row in the data list, so replicates line up laterally across columns)



DEFINE DATA LIST PAGE, WITH WORKSHEET AND DATA LIST ENTRIES

3. To add some, but not all, worksheet entries to the data list, click and drag or press Shift and the Up or Down arrow keys to select contiguous rows. Once they are highlighted, click the **Add Selected Plates** button in the middle of the page.

*Note: If you want to add non-contiguous MicroPlates, click the **Add Selected Plates** button separately for each contiguous group selected. The groups are added cumulatively to the data list.*

4. To delete MicroPlates from the data list, select the wells you want to delete, then click **Delete Selected Plates**.

5. To clear all entries from the data list (you must do this to begin creating a new data list), click **Clear all Plates**.

6. If you've already created and saved a data list and wish to recall that list, click **Load** at the bottom left of the page. All entries existing at the time you last saved will appear in the data list.

7. If you have added or deleted MicroPlates from the data list, you may want to recalculate the average plot of the replicates. To do so, click **Average Replicates** at the bottom of the page. If you encounter any replicates with a Plate Revision Letter that is different from the first one, a warning will appear. Click **OK** for each warning. A progress bar will show the advancement of the averaging and plotting process.

8. When you are satisfied with the results of your search (as displayed in the data list), click Save to retain the data list for future use and/or parametric analysis. A Windows Save File dialog pops up. Name the data list and click **OK** to save it in the desired directory. The program will automatically append a .dlb file extension to the data list's file name.

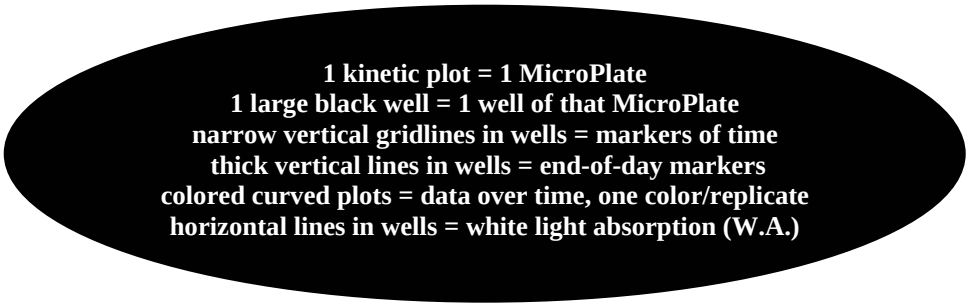
OMNILOG PM FILE EXTENSIONS

.oka	new 11-field data file name
.dlb	data list
.w4c	worksheet list
[01-25][a,b]	old 4-field data file name
.cvs	comma delimited CSVfile

Displaying Kinetic Plots

A kinetic plot shows each well in a MicroPlate in a data list. These plots are graphical representations of the data contained in a data list.

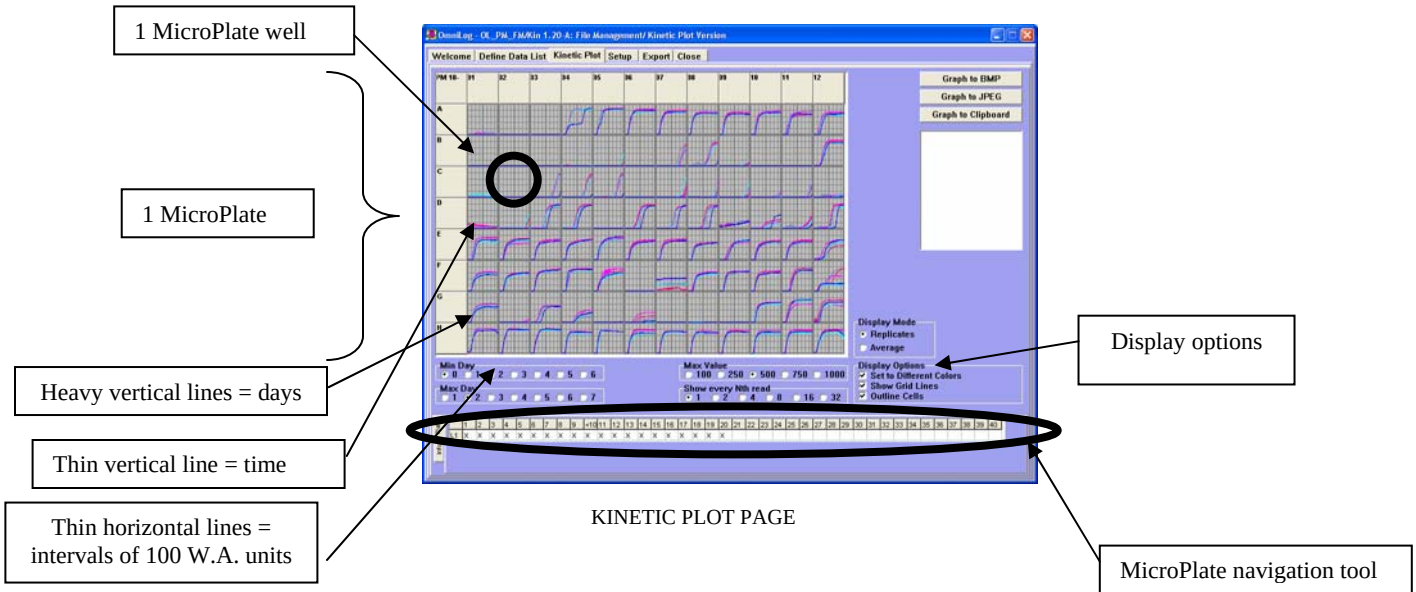
Kinetic plots represent a change in experimental conditions in wells over time. This change reflects a combination of growth and respiration of the test organism.


1 kinetic plot = 1 MicroPlate
1 large black well = 1 well of that MicroPlate
narrow vertical gridlines in wells = markers of time
thick vertical lines in wells = end-of-day markers
colored curved plots = data over time, one color/replicate
horizontal lines in wells = white light absorption (W.A.)

1. Before navigating to the kinetic plot page, you can set up the colors you want to appear for each column of data. The color bars at the top of each column in the data list dictate which colors will appear on the kinetic plot page. If you want to change these colors, click on the color bar at the top of each data list column. A **Color** Dialog pops up. Select the desired color by clicking on that color, then click **OK**.

COLOR SELECTION DIALOG

2. Select a MicroPlate type you'd like to begin with in the data list (on the **Define Data List** page) by selecting that row.
3. Click the **Kinetic Plot** tab on the menu bar. The wells will fill with graphical data. Wait until the entire display appears before clicking anything else.
4. Visually scan the display for gross experimental errors:
   - Flat lines indicate no growth. Look for flat lines in wells where you would expect growth.
   - Look for growth (graphical data) in unexpected wells.
5. Visually scan the color plots for indications of experimental reproducibility. The display can show up to 10 colors (replicates in excess of 10 are still displayed, but not in different colors). The closer those colored lines lie on top of each other, the greater the reproducibility.
6. The software allows you to view graphical data in a number of ways:


1 MicroPlate well
1 MicroPlate
Heavy vertical lines = days
Thin vertical line = time
Thin horizontal lines = intervals of 100 W.A. units
Display options
MicroPlate navigation tool
Plate tab: Click to display different MicroPlates
Info tab: change graphical colors here

KINETIC PLOT PAGE

MICROPLATE NAVIGATION TOOL

KINETIC PLOT DISPLAY OPTIONS

Option	What it does	What to Click
MicroPlate navigation tool	allows you to select which MicroPlate in the current data list you want to view graphically; small carets appear immediately to the left and right of the displayed file.	click on the number of the MicroPlate you want to view (**1-40**)
Min Day/Max Day	allows you to narrow the span of days displayed (for example, if your experiment spanned 7 days, but there was no growth on days 1-3 and excessive growth on days 6 and 7, you can choose to view only days 4-5 if desired)	click the radio button desired for each of **Min Day** and **Max Day**
Max Value	adjusts the vertical scale of the display; you can adjust this value to show the plots on a maximal scale without being out of range	click the desired radio button for **Max Value**

Option	What it does	What to Click
Show Every Nth Read	this adjustment effects the speed at which the display changes; the higher the number you select, the faster the graphical display changes. This occurs because of the elimination of some data points to increase speed.	click the desired radio button for **Show every Nth read**
Set to Different Colors	allows you to display each replicate's plot in a different color.	check the **Set to Different Color** box
Show Grid Lines	allows you to add or remove the horizontal and vertical grid lines within a well	check the **Show Grid Lines** box
Outline Cells	allows you to add or remove the dark outline around each well	check the **Outline Cells** box
Replicate Display Mode	shows the kinetic plots for all the replicate plates	click on the **Replicates** radio button
Average Display Mode	shows the average kinetic plot and the standard deviation (SD); the average + SD is displayed as shading on the upper edge of the curve, while the average – SD is displayed as shading on the lower edge of the curve.	click on the **Average** radio button

Averaging Data

In order to perform parametric analysis on the data you've collected, you need to average the replicates in your data list. The program does these averages automatically every time you save a data list. You may want to experiment with various settings that the affect this averaging.

To average replicates:

1. Navigate to the **Define Data List** page by clicking on the proper menu tab.
2. Click the **Average Replicates** button at the lower edge of the page. (If the averages have already been calculated by the program, you will see a message indicating this.)
3. Navigate to the **Kinetic Plot** page by clicking on the proper menu tab.
4. Click on the **Average** radio button on the right lower side of the page. The averages will be displayed as a black line in each well.

A1-zeroing the data

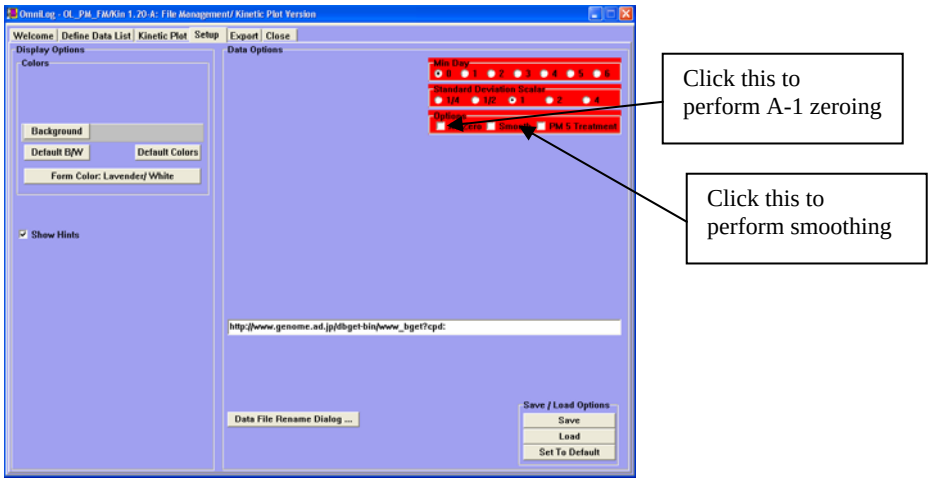
PM MicroPlates 1-8 have a negative control well (containing no chemical) at the A-1 position. If you have a plate that you suspect contains a background color in all wells, you may want to subtract the color in A-1 from all wells. This will clear the suspected background color from all wells. In general, you'll do this at the same time you average your data.

> **Warning:** Excessive growth in well A1 prevents interpretation of the MicroPlate and the usefulness of the data. A1-zeroing is only for small corrections.

1. Navigate to the **Setup** page by clicking on the proper menu tab.
2. In the upper right corner, you will see a red box.
3. Check the **A1-Zero** box.
4. Navigate to the **Define Data List** page by clicking on the proper menu tab.
5. Click the **Average Replicates** button.

*Note: Even if your data list does not have more than one replicate, you must click **Average Replicates** in order to A1-Zero the data.*

6. The software will display a message asking if you want to proceed with averaging replicates. Click **Yes** or **No**, as appropriate.
7. Averaging will NOT automatically save your data. Use Save to insure saving your data.



Smoothing the Data

A kinetic plot may have optical noise. If desired, you can smooth out the data to create a more even line. The Smooth function of the software recalculates each OmniLog Signal value for each read as a weighted average of two consecutive reads. This results in a smoother plot, which makes plot comparison easier (between replicates, for example). This selection is a matter of personal preference. In general, you'll do this at the same time you average your data.

Note: Smoothing data influences parametric analysis results.

1. On the **Setup** page, click the **Smooth** radio button in the upper right corner of the page.
2. Navigate to the **Define Data List** page by clicking the proper menu tab.
3. Click the **Average Replicates** button.

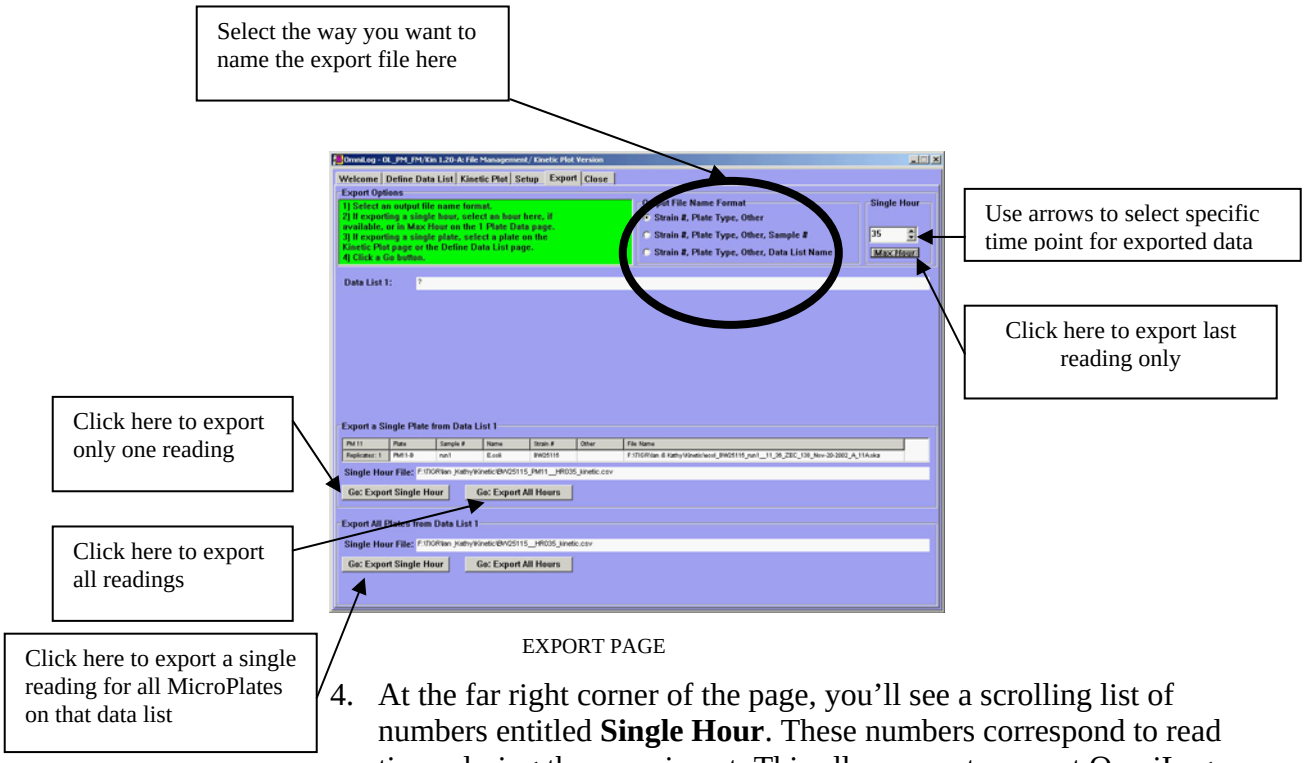
*Note: Even if your data list does not have more than one replicate, you must click **Average Replicates** in order to smooth the data.*

4. You will see a message reminding you that you have selected a non-default option.

Exporting Data

OmniLog PM software allows you to export large quantities of data from kinetic plots to an external program such as Excel. The raw data is exported in units of OmniLog Signal.

1. Make sure the data you want to export is displayed on-screen in the kinetic plot. Check the MicroPlate navigation tool across the bottom of the page to make sure the desired MicroPlate is shown; you'll see small carets on either side of the selected plate number (such as <7>).
2. Click the **Export** tab on the menu bar.
3. The upper right side of the page contains three radio buttons that allow you to select a naming format for the export file. This information is selected from the 11-field data file name for the first replicate of the selected MicroPlate. Select the desired format by clicking on the proper radio button. As you click each one, you'll see changes in the export file name listed in the **Single Hour File** display box.


Select the way you want to name the export file here
Use arrows to select specific time point for exported data
Click here to export last reading only
Click here to export only one reading
Click here to export all readings
Click here to export a single reading for all MicroPlates on that data list
Welcome
Define Data List
Kinetic Plot
Setup
Export
Close
Export Options
Single Hour
Max Hour
Data List 1:
Export a Single Plate from Data List 1
Single Hour File:
Go: Export Single Hour
Go: Export All Hours
Export All Plates from Data List 1

EXPORT PAGE

4. At the far right corner of the page, you'll see a scrolling list of numbers entitled **Single Hour**. These numbers correspond to read times during the experiment. This allows you to export OmniLog Signal values for specific time points (including an end-point reading). Click the **Up** and **Down** arrows to select the desired time point. Click the **Max Hour** button if you want to export only the last reading.
5. Observe the **Data List 1** field. If you've saved the data list, the name will appear here; if not, this field will be empty. You can export the data whether you've saved the data list or not.
6. To export data for the hour you selected in the upper right corner, click **Go: Export Single Hour**.
7. To export data for all hours in the displayed MicroPlate, click **Go: Export All Hours**.
8. The bottom section of the Export page **(Export All Plates from Data List 1)** allows you to export data for all MicroPlates in the data list you are currently working with.
    - Click **Go: Export Single Hour** (the one on the bottom of the page) to export the data for the hour selected in the upper right corner (but for all MicroPlates in the current data list).
    - Click **Go: Export All Hours** (the one on the bottom of the page) to export all values over the entire experiment (for all MicroPlates in the current data list).

9. A progress bar and a message to “Please Wait” will appear while the data is exported.
10. To locate the exported data, open Excel and find the file as it is named in the Single Hour File display box. All OmniLog Signal values for the selected readings will appear on a spreadsheet.
11. Click the Close tab on the menu bar when you’ve finished. Click Close again to exit the program.

3. Comparing Data Lists and Generating Reports

In this section:
- ➔ **Comparing Data Lists**
- ➔ **Saving Graphical Displays and Viewing Saved Files**
- ➔ **Generating Non-Graphical Reports**
- ➔ **Exporting Parameter Values**

The Parametric portion of the OmniLog PM software displays a comparison of the data in two data lists. The program superimposes two kinetic plots, allowing you to identify anomalous wells easily. This is useful for comparing a compound-treated organism with a reference organism. You can also compare the mutant form of an organism with the wild type of that organism. The contrasting colors used for the superimposed curves visually guide you in identifying wells of interest.

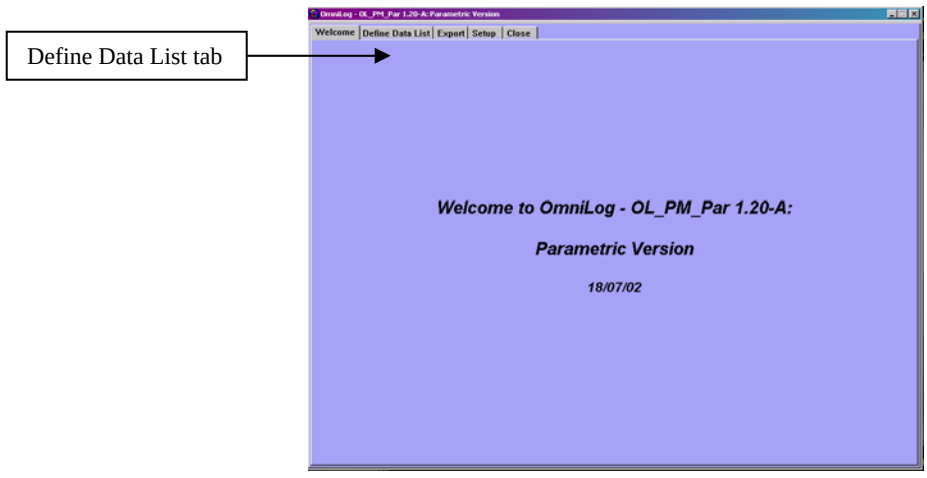
Once you've displayed meaningful visual data, you can save it and generate reports. These reports include graphical summaries of data (kinetic plots) exportable into Word® or Power Point® documents, as well as non-graphical data summaries you can export into Excel® or Access®.

Comparing Data Lists

Once you've created at least two data lists using the File Management/Kinetic Plot portion of the software, you will open the Parametric program and begin the process of creating a visual display that best serves your experimental purposes.

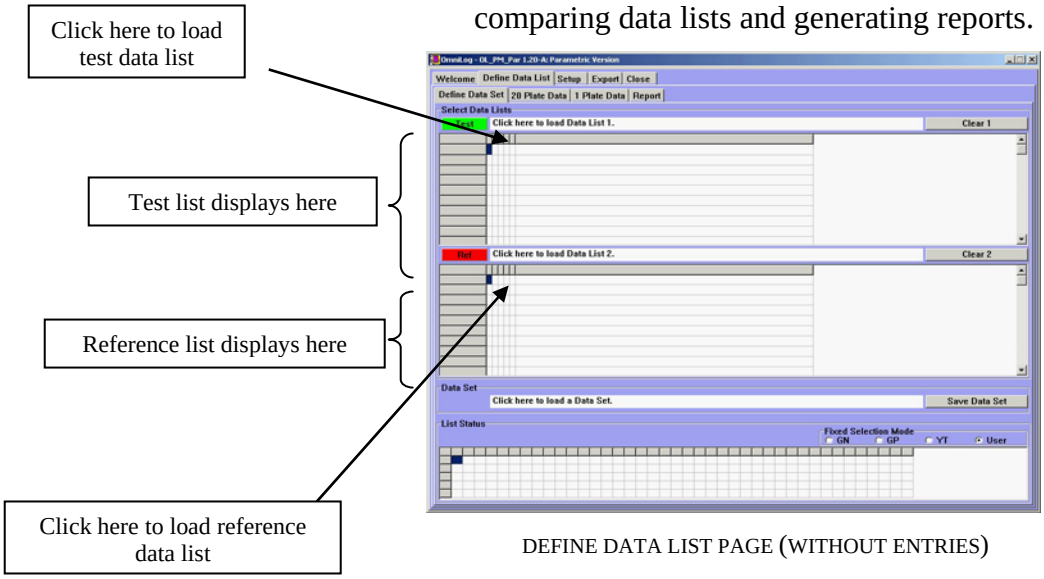
Retrieving test and reference data lists

1. Double-click on the OL_PR_12.exe icon in the proper OmniLog PM folder. A **Welcome** page appears, displaying the program name, version, and date.



WELCOME PAGE (PARAMETRIC PROGRAM)

2. Click the **Define Data Lists** tab on the menu bar. The page that appears has additional tabs you'll use during the process of comparing data lists and generating reports.



DEFINE DATA LIST PAGE (WITHOUT ENTRIES)

3. Click in the **Click here to load Data List 1** white box at the top of the page (adjacent to the green box) to load the test data list (this will serve as the test list for this data set).
4. Navigate through the directories until you find the desired data list; highlight the name and it will appear in the **File Name** box. These files have a .dlb extension.
5. Click **Open**.

Note: Check data file names carefully. Make sure you retrieve the proper data files in the upper half of the page and the proper data files in the lower part of the page. If you load a reference file on the top and a test file on the bottom, the mathematical calculations will be incorrect.

6. The program will automatically calculate the parameters and populate the upper half of the page with the retrieved data list. Allow a few moments for this to occur.
7. Click in the **Click here to load Data List 2** white box in the middle of the page (adjacent to the red box) to load the reference data list (this will serve as the reference list for this data set).

Navigate through the directories until you find the desired data list; select the name and it will appear in the **File Name** box of the Open File Dialog. These files have a .dlb extension.

8. Click **Open**.
9. The program will automatically calculate the parameters and populate the lower half of the page with the retrieved data list. Allow a few moments for this to occur.

Note: The columns that appear on the test and reference data lists are the same as those that appeared in the data list grid created using the Kinetic module of the program.

WHAT'S ON THE DISPLAYED DATA LISTS?

Plate Type (Left Column):

⇒ Plate Type (unique identifier assigned to each kind of PM MicroPlate, depending on the chemicals contained in all of the 96 wells)

⇒ This column lists a subset of the 40 possible PM MicroPlate types, depending on the MicroPlates used in that experiment

Color Bars:

⇒ Green **Test** bar in upper section denotes data list for test (mutant) form of organism

⇒ Red **Ref** bar in upper section denotes data list for reference (wild-type) form of organism

Expanding Columns:

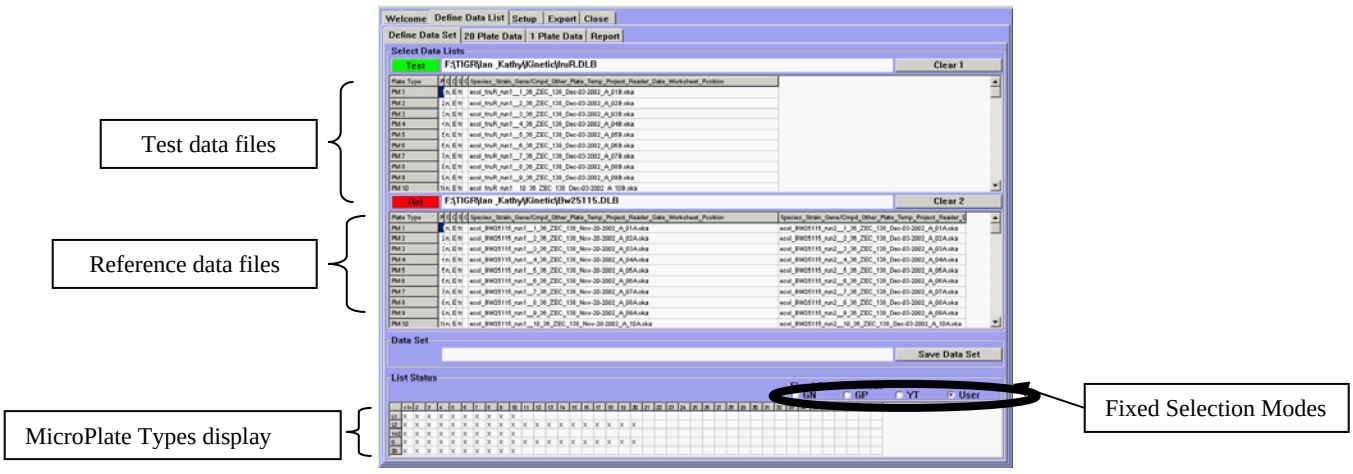
⇒ These five expandable columns list the most common search criteria for MicroPlates (position, sample number, strain name, strain number, and other)

⇒ To view fully, click on the edge of the header row and drag column to right

Data File Columns:

⇒ These wide columns list the 11-field names of the files you created in the data list

⇒ Each additional column represents an additional replicate if that plate has an additional replicate



DEFINE DATA LISTS PAGE (WITH TEST AND REFERENCE LISTS)

Narrowing down the types of MicroPlates displayed

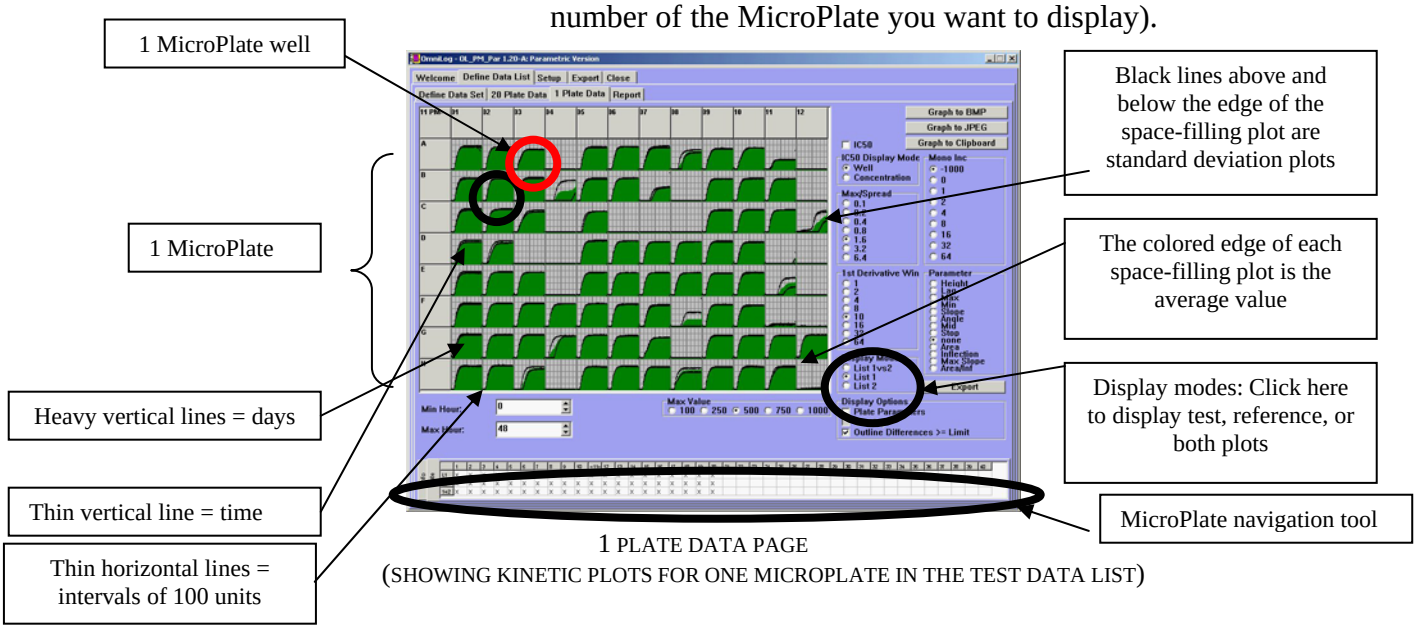
1. Once both data lists (test and reference) are displayed, use the MicroPlate Types display tools at the bottom of the page to define the types of MicroPlates you want to compare.
    - In the **Fixed Selection Mode** groupbox, click **GN** (gram negative) to display MicroPlates PM 1-20
    - In the **Fixed Selection Mode** groupbox, click **GP** (gram positive) to display MicroPlates PM 1-10 and 31-40
    - In the **Fixed Selection Mode** groupbox, click **YT** (yeast) to display MicroPlates PM 1-10 and 21-30
    - In the **Fixed Selection Mode** groupbox, click **User** to display the first 20 MicroPlate types in each data list
2. At the bottom of the page, click on the **List Status** grid to select which MicroPlate type you want to show in the kinetic plots.
    - Row **L1** displays every MicroPlate type available in the test data list
    - Row **L2** displays every MicroPlate type available in the reference data list
    - Row **L1 & L2** displays only MicroPlate types contained in BOTH lists
    - Row **S** to displays only those you selected in Fixed Selection Mode
    - Row **20** displays only the MicroPlate types common to the test list, the reference list, and Fixed Selection Mode

Viewing single MicroPlate comparative plots

Comparing test and reference plots one MicroPlate at a time is useful for selecting the specific parameters by which you want to characterize your data and for visually inspecting superimposed plots to determine a desirable threshold. The **1 Plate Data** page is the place where you will refine the specifics of your plots and parameters.

1. Click the **1 Plate Data** sub-tab on the menu bar. The program displays the kinetic plots for the test data list by default. You'll note that the **List 1** radio button is selected in the **Display Mode** groupbox, which corresponds to the test data list.

- Click the **List 2** radio button to display one MicroPlate from the reference data list.
- Click the **List 1vs2** radio button to display superimposed plots and areas of overlap between the test and reference data.
- Use the MicroPlate navigation tool across the bottom of the page to move from one MicroPlate to another (click on the number of the MicroPlate you want to display).


1 MicroPlate well
1 MicroPlate
Heavy vertical lines = days
Thin vertical line = time
Thin horizontal lines = intervals of 100 units
Black lines above and below the edge of the space-filling plot are standard deviation plots
The colored edge of each space-filling plot is the average value
Display modes: Click here to display test, reference, or both plots
MicroPlate navigation tool

1 PLATE DATA PAGE
(SHOWING KINETIC PLOTS FOR ONE MICROPLATE IN THE TEST DATA LIST)

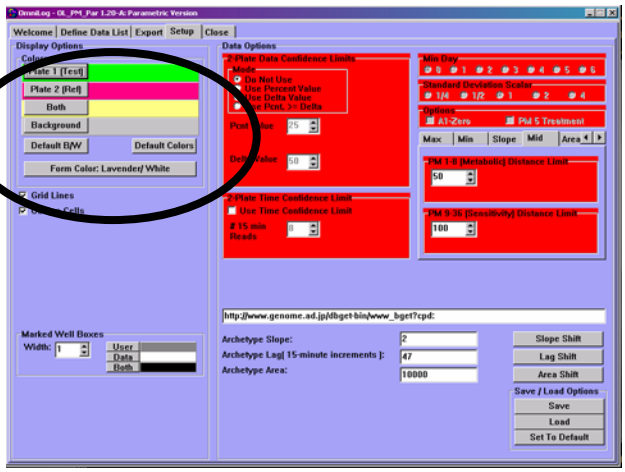
1 entire grid = 1 MicroPlate
one 8 x 12 well = 1 well of that MicroPlate
narrow vertical gridlines in wells = markers of time
thick vertical lines in wells = end-of-day markers
colored, space-filling plots = average data over time
wiry black lines above and below curves = ± SD
horizontal lines in wells = white light absorption (W.A.)

2. All plots are displayed in default colors (green for test plots, red for reference plots, and yellow for superimposed areas in common). To change these colors, click the Setup tab on the menu bar. Go to the upper left corner of the page to change plot and background colors.
    - In **Display Options - Colors**, click **Plate 1 – Test**. A color dialog pops up. Click on the desired color for the test plots, then click **OK**.

The graphical plots shown on the 1 Plate Data page are AVERAGED plots of replicates.

- In **Display Options - Colors**, click **Plate 2 – Ref**. A color dialog pops up. Click on the desired color for the reference plots, then click **OK**.
- In **Display Options - Colors**, click **Both**. A color dialog pops up. Click on the desired color for the areas in common, then click **OK**.
- In **Display Options - Colors**, click **Background**. A color dialog pops up. Click on the desired color for the grid areas behind the plots, then click **OK**.
- In **Display Options - Colors**, click **Default B/W**. All plots will automatically revert to black, white, and gray (this can be useful for black and white printing).
- In **Display Options - Colors**, click **Default Colors**. All plots will automatically revert to green (test), red (reference) and yellow (both).
- In **Display Options - Colors**, click **Form Color – Lavender/White**. This allows you to toggle the background color of the entire program page from pale purple to white.

Change display colors here



SETUP PAGE

COLOR SELECTION DIALOG

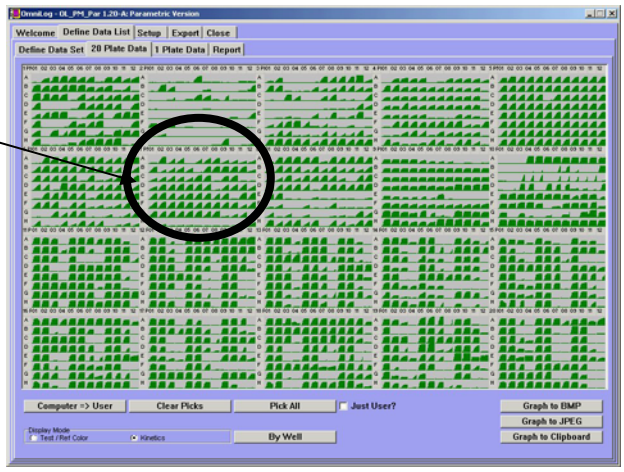
3. Use the **MicroPlate navigation tool** (**List Status)** across the bottom of the page to select which MicroPlate number you want to display. Small carets appear immediately to the left and right of the selected number.

Viewing multiple MicroPlates

The software also gives you the option of viewing 20 MicroPlates types at once. This enables you to easily scan many kinetic plots and see patterns of differences and similarities. These distinctions stand out because the list and reference data plots are superimposed over each other.

As you work with your data, you will likely navigate frequently between the **1 Plate Data** page to the **20 Plate Data** page.

1. Click **20 Plate Data** tab on the menu bar.
2. Right-click on any well to view a description of the chemical contained in that well, along with the mode of action.
3. Double-click on any of these descriptions to go directly to the on-line KEGG website.

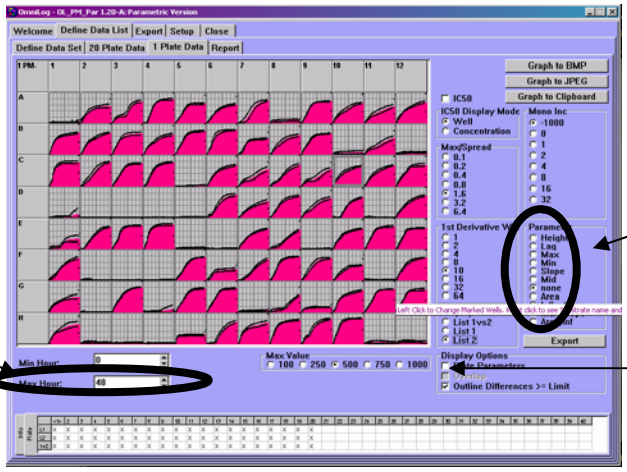


20 PLATE DATA PAGE

Selecting parameters

OmniLog PM enables you to reduce the multitude of data on your data lists by extracting specific parameters that focus on one or more aspects of the plot, depending on your areas of interest. The parameters are numerical characteristics of the plots displayed. Selecting specific parameters helps you find out more about the characteristics of an organism, as well as conduct statistical analyses of these parameters.

The most commonly selected parameters are described here; see "Advanced Functions" for descriptions of the other parameters.


Click one of these radio buttons to select a specific parameter
Adjust this down a few hours to eliminate plot anomalies if necessary
Check this checkbox to display parametric or derivative plots

1 PLATE DATA PAGE

(SHOWING KINETIC PLOTS FOR ONE MICROPLATE ON THE REFERENCE DATA LIST)

MOST COMMONLY SELECTED PARAMETERS

Parameter	What it is	What to Click
Height	• the average height of the plot, in white light absorption values • the blue lines represent a 'parametric plot,' which is a graphical representation of the minimum value, maximum value, lag time and plateau time parameters	click on the **Average** radio button in the **Parameter** groupbox
Area	• the sum of all OmniLog values over all timepoints • the blue lines represent the same parametric plot described above	click on the **Area** radio button in the **Parameter** groupbox
Inflection	• the time at which there is an equal area under the derivative curve on both sides • the blue line represents the first derivative plot • peaks indicate where the bacteria grew most rapidly.	click on the **Inflection** radio button in the **Parameter** groupbox

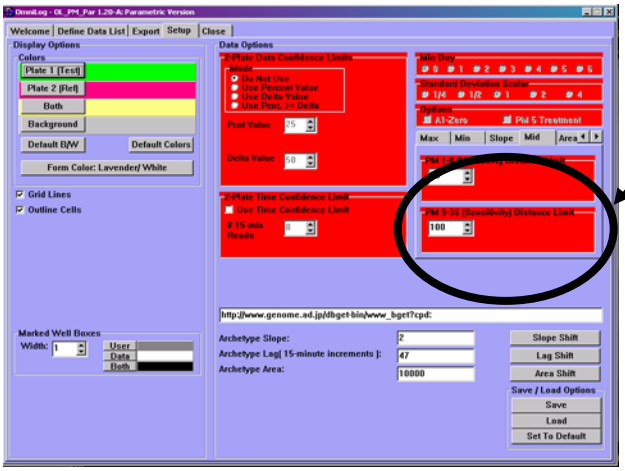
1. Click the radio button for the desired parameter. Keep the following in mind:
    - The Average Height and Area parameters are the most stable and well-behaved parameters. They have the disadvantage, however, of being sensitive to the accumulation of reads in excess of the Plateau Time. Consequently, it is advisable to adjust the Max Hour so that it is only slightly greater than the Plateau Time.
    - The Inflection Time parameter is sensitive to anomalous upspikes in the plot. When selecting this parameter, you might want to reset the Max Hour designation for the experiment to a lower number. This will get rid of any anomalies in the plots (noise in the curves) and allow you to look at only a proscribed time period of the study. If, for example, the entry under **Max Hour** is 48, reducing it to 42 will eliminate any undesired noise during the period between 42 and 48 hours.

Setting a threshold

The software allows you to set a threshold (value of significance) as you analyze your data set. Thresholds provide a rapid way to select wells that are significantly different, while excluding insignificant ones.

Setting a threshold is an art, interive process. What you view as a "strikingly different" will vary from experiment to experiment.

1. Display the desired MicroPlate on the **1 Plate Data** page.
2. Select the desired parameter, for example **Height**.
3. Look for wells with strikingly different plots. For example, a well might have a reference plot with a height of 211 and a height of 33 (a difference of 178).
4. Once you have a numerical value in mind for what you consider a significant difference for that data set, click the **Setup** tab on the menu bar.

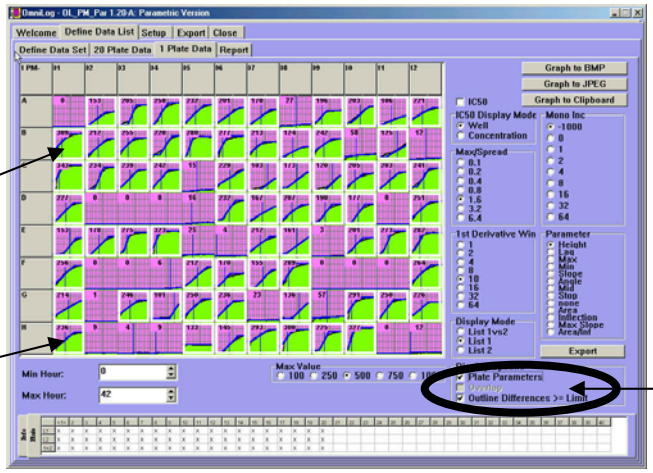


SETUP PAGE

5. At the right of the page, you'll see the **Distance Limit** edit boxes. The PM 1-8 distance limit covers metabolic MicroPlates, while the PM 11-36 distance limit covers sensitivity MicroPlates. Use the Up and Down arrows to adjust these numbers as desired. Remember, the purpose of changing the threshold is to create a more obvious visual aid to help you identify wells of scientific interest. You may, for example, want to set the PM 1-8 limit at 100 and the PM 11-36 limit at 150.
6. As soon as you start to adjust the numbers, the boxes will flash red. Press **Enter** once you reach the number you want in each box.
7. Click the **Define Data List** on the menu bar, then the **1 Plate Data** sub-tab. Check the **Outline Differences** options under the Display Options group box.

The outlined wells will change based on the thresholds you set. If necessary, go back and forth between pages, adjusting the thresholds until you are satisfied with the resulting outlined wells.

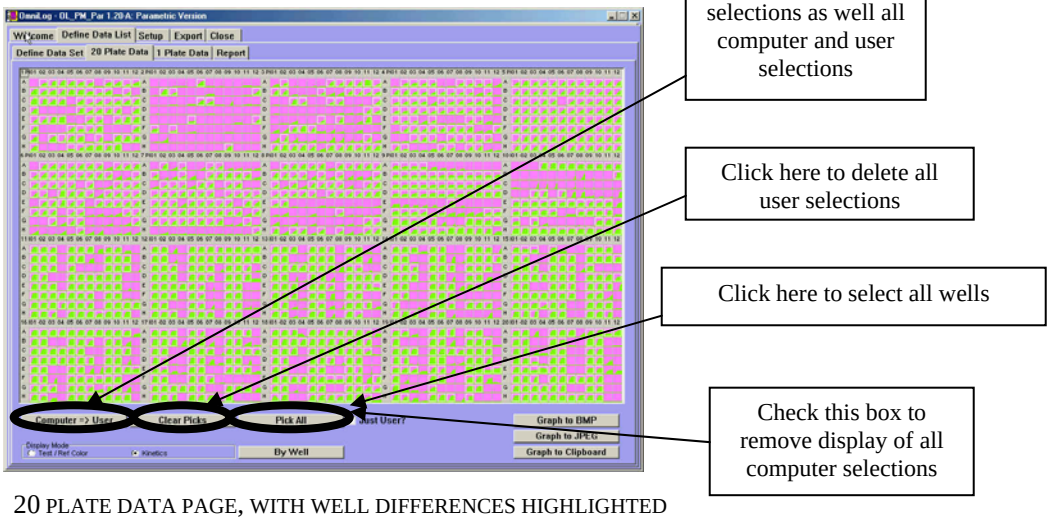
8. On the **1 Plate Data** page, click **Outline Differences** in the **Display Options** groupbox. This gives you a quick visual way to outline wells that are different. Use the **MicroPlate navigation tools** across the bottom of the page to display other MicroPlates in that data set. The same thresholds, parameters, and display option you selected will show for every MicroPlate.


Wells with white outlines mark differences based on threshold settings
Spindly blue lines appear when Plate Parameters option is enabled
Click here for visual aids that show differences in wells

1 PLATE DATA PAGE, WITH WELL DIFFERENCES HIGHLIGHED

The white boxes that outline wells are tentative until you're completely satisfied with the thresholds and parameters you've selected. You then have to save your selections by using the functions at the bottom of the 20 Plate Data page.

9. Click the **20 Plate Data** tab to view all 20 MicroPlates at once, with the white outlines giving you a sweeping view of which wells might be interesting across the entire experiment.


Click here to make selections as well all computer and user selections
Click here to delete all user selections
Click here to select all wells
Check this box to remove display of all computer selections

20 PLATE DATA PAGE, WITH WELL DIFFERENCES HIGHLIGHTED

Accepting or customizing selections

The white boxes that outline the differences between wells are very useful markers. The software enables you to either accept the program's automatic selections (white boxes) or manually change the boxed selections until you are satisfied with the data captured.

1. Make sure you are on the **20 Plate Data** page.
2. If you are satisfied with the thresholds you've entered and with the white boxes the program outlined based on your selected parameters and thresholds, click the **Computer → User** button at the bottom of the **20 Plate Data** page. The white outlines will all turn black, signifying that they are no longer tentative.

The reason for setting parameters, thresholds, and for carefully selecting and/or deselecting wells is to create a useful set of data for display and for creating a report for exporting to Excel or Access.

3. If you are not fully satisfied with the computer's selections, you can manually select or deselect individual wells, as follows:
    - Work on either the **1 Plate Data** page or the **20 Plate Data** page.
    - Make sure the **Outline Differences** checkbox is checked.
    - If you have NOT clicked **Computer → User**, you can select or deselect wells by clicking on them. Wells that the computer has already outlined white will turn black, signifying that you definitely want to select those wells. If you click a black-outlined well again, it will turn back to white (deselecting it).
    - If you HAVE clicked **Computer → User** (saving the computer's selections), you can deselect individual wells by clicking on them. The black outlines will turn white.
    - If you see wells that the computer did not select, you can select them by clicking on them. The outlines will turn gray. These are called "user picks." If you want to display ONLY these (not the computer's choices), check the **Just User** checkbox. All outlines will disappear except the gray ones you selected manually.
4. If desired, click **Clear Picks** on the **20 Plate Data** page to go back to the original computer selections, with no user selections outlined.
5. If desired, click **Pick All** on the **20 Plate Data** page to select ALL wells for inclusion in your data set. All wells will be outlined black or gray, indicating selection.

WHAT DO DIFFERENT COLORED WELL OUTLINES INDICATE?

White-Outlined Wells:

⇒ OmniLog PM software has performed calculations and selected these wells for inclusion in your data set. They are tentative selections until you save them.

Black-Outlined Wells:

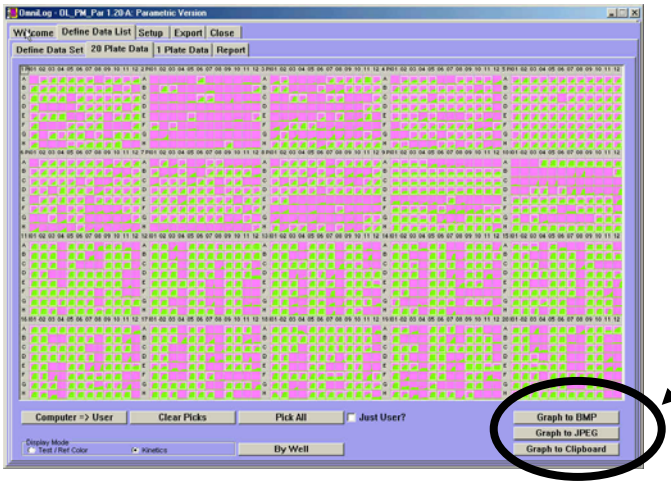
⇒ You have indicated that the computer's selection is also one that you would make manually for definite inclusion in the data set.

Gray-Outlined Wells:

⇒ You have selected wells that the software did not select. They will be included in your data set.

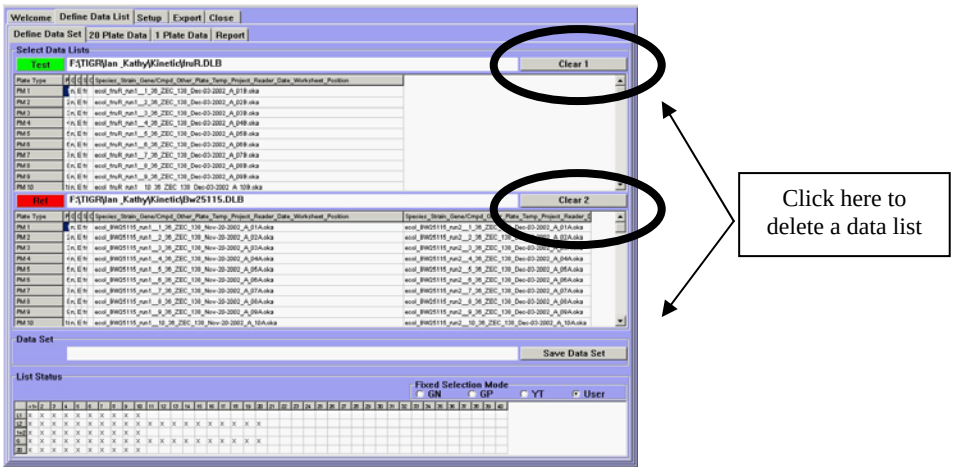
Saving graphical displays and viewing saved files

1. Once you are satisfied with the parameters you've selected, the set thresholds, and the wells selected for inclusion in the data set, click one of the following:
   - To save the displayed plot as a bitmap, click **Graph to BMP** at the lower right corner of the **20 Plate Data** page. Name your file as desired and save to a selected directory by clicking **Save**.
   - To save the displayed plot as a .jpeg, click **Graph to JPEG** at the lower right corner of the **20 Plate Data** page. Name your file as desired and save to a selected directory by clicking **Save**.
   - To copy the displayed plot directly into the clipboard, click **Graph to Clipboard** at the lower right corner of the page.



20 PLATE DATA PAGE

2. View a previously saved data set, as follows:
   - Click the **Define Data Set** tab on the menu bar.



DEFINE DATA SET PAGE

   - Click **Clear 1** and **Clear 2** to delete the test and reference data lists.
   - Click on the white box containing the words **Click here to load data list 1** and **Click here to load data list 2**. Retrieve the desired files from the Windows open file dialog. The files will contain all your previous settings, thresholds, and selected wells.
   - Alternatively, you may save two data lists as a data set by clicking the **Save Data Set** button and using the pop-up **Save File Dialog**. The program will automatically append a .dsb extension to the data set file. Subsequently, you can load the data set by clicking the **Load Data Set** box and using the pop-up **Open File Dialog**. The two data lists comprising that data set will automatically be loaded.

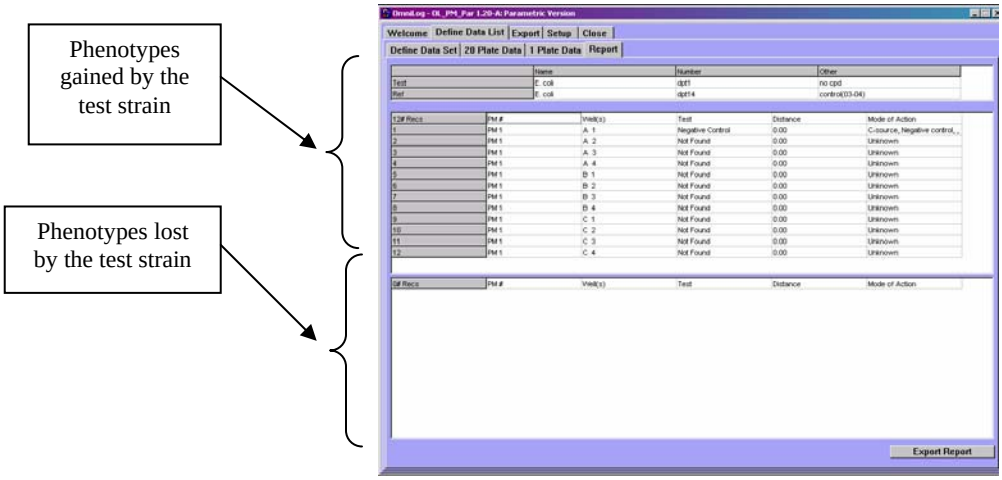
Generating non-graphical reports

The software allows you the option of displaying your parameter values in spreadsheet format for easy, thorough review. You can also save these spreadsheets as Excel .csv files.

1. Select a parameter on the **1 Plate Data** page.
2. Click the **Report** tab on the menu bar.

3. The parameter values of the user-selected wells on the **1 Plate Data** and **20 Plate data** pages will be transcribed into spreadsheet format.
4. Review the spreadsheet for the following:
    - Check the top of the report for organism strain name, strain number, and any other information you may have included for the first replicate of the test and reference data lists.
    - Check the midsection of the list for phenotypes gained. For these, the test had a greater value than the reference for the parameter selected.
    - Check the bottom of the report. This displays phenotypes lost by the test strain. These losses correspond to wells you selected that had a negative value for difference (test list average height – reference list average height). In other words, the reference had a greater value than the test for the parameter selected.

Note: These values will fall above the thresholds you set for that experiment.



SPREADSHEET REPORT

5. To export this information into Excel, click the **Export Report** button at the bottom right corner of the page. Name the file as desired and click **Save** to save as a .csv file.

Report Date : 4/14/2003 10:39:20 AM

	Name	Strain Nu	Other
Test	E. coli	dpt20(2)	control (20&21)
Ref	E. coli	dpt01	

Phenotypes Gained - Faster Growth / Resistance

PM	Wells	Test	Differenc	Mode of Action
PM14	A11	Sanguinari	177.37	ATPase, Na+/K+ and Mg++
PM14	C06	1-Hydroxy	232.98	chelator, lipophilic
PM14	G06	Potassium	155.04	oxidizing agent
PM19	G06, G07,	Dihydrostr	602.44	protein synthesis, aminoglycoside
PM16	E03, E04	Streptomy	458.67	protein synthesis, aminoglycoside
PM11	A04	Amikacin	158.06	protein synthesis, aminoglycoside
PM13	E08	Geneticin	157.18	protein synthesis, aminoglycoside
PM14	D02, D03	Cadmium	377.65	transport, toxic cation
PM14	F06, F07	Piperacilli	430.77	wall, lactam

Phenotypes Lost - Slower Growth / Sensitivity

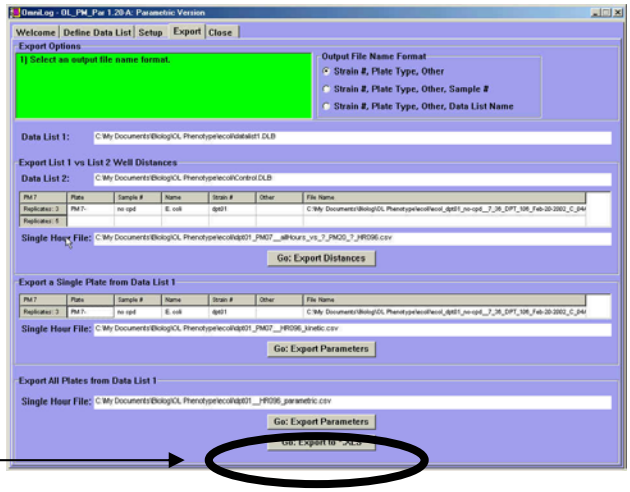
PM	Wells	Test	Differenc	Mode of Action
PM01	A07	L-Aspartic	-248.96	C-source, amino acid
PM01	D01	L-Asparag	-162.74	C-source, amino acid
PM01	A12	Dulcitol	-208.59	C-source, carbohydrate
PM01	A05	Succinic A	-176.07	C-source, carbohydrate
PM02	F03	Melibionic	-161.93	C-source, carbohydrate
PM01	G11	D-Malic A	-268.62	C-source, carboxylate
PM01	G12	L-Malic Ac	-247.96	C-source, carboxylate
PM01	F05	Fumaric A	-246.13	C-source, carboxylate
PM01	C03	D,L-Malic	-241.4	C-source, carboxylate

PRINTOUT OF PARTIAL PAGE OF SPREADSHEET REPORT IMPORTED INTO EXCEL

Exporting parameter values

OmniLog PM enables you to export your parameter values into Excel as multi-worksheet .xls files. This is done one data list at a time. These worksheets organize the parameters according to MicroPlate number or display format.

1. With the data list to be exported loaded as Data List 1 (also called the test data list) in the **1 Plate data** page, select the **Max Hour** setting desired.
2. Click the **Export** tab on the menu bar.
3. Click the **Go: Export to XLS** button at the bottom of the page.



Click here to export a parameter values list into multi-worksheet .xls file

EXPORT PAGE

4. Name and save the file as desired.

Plate	Replicate	Well	Chemical	Mode of Action	Maximum	Minimum Height Well 0	Minimum Height Well 1
					168.00	500.00	500.00
PM11	average	A01-A04	Amikacin	protein synthesis, ami	46.00	16.00	0.00
PM11	average	A05-A08	Chlortetra	protein synthesis, tetr	46.00	16.00	0.00
PM11	average	A09-A12	Lincomyci	protein synthesis	46.00	16.00	0.00
PM11	average	B01-B04	Amoxicilli	wall, lactam	46.00	16.00	0.00
PM11	average	B05-B08	Cloxacillin	wall, lactam	46.00	16.00	0.00
PM11	average	B09-B12	Lomefloxa	DNA topoisomerase,	46.00	16.00	0.00
PM11	average	C01-C04	Bleomycin	DNA polymerase	46.00	16.00	0.00
PM11	average	C05-C08	Colistin	membrane, cyclic pep	46.00	16.00	0.00
PM11	average	C09-C12	Minocyclin	protein synthesis, tetr	46.00	16.00	0.00
PM11	average	D01-D04	Capreomy	respiration, Na+-K+ A	46.00	16.00	0.00
PM11	average	D05-D08	Demecloc	protein synthesis, tetr	46.00	16.00	0.00
PM11	average	D09-D12	Nafcillin	wall, lactam	46.00	16.00	0.00
PM11	average	E01-E04	Cefazolin	wall, cephalosporin	46.00	16.00	0.00
PM11	average	E05-E08	Enoxacin	DNA topoisomerase,	46.00	16.00	0.00
PM11	average	E09-E12	Nalidixic a	DNA topoisomerase	46.00	16.00	0.00
PM11	average	F01-F04	Cefotaxim	wall, cephalosporin	46.00	16.00	0.00
PM11	average	F05-F08	Erythromy	protein synthesis, mac	46.00	16.00	0.00
PM11	average	F09-F12	Neomycin	protein synthesis, ami	46.00	16.00	0.00
PM11	average	G01-G04	Ceftriaxon	wall, cephalosporin	46.00	16.00	16.00
PM11	average	G05-G08	Gentamici	protein synthesis, ami	46.00	16.00	0.00
PM11	average	G09-G12	Norfloxaci	DNA topoisomerase,	46.00	16.00	0.00
PM11	average	H01-H04	Cephalothi	wall, cephalosporin	46.00	16.00	1.00
PM11	average	H05-H08	Kanamyci	protein synthesis, ami	46.00	16.00	0.00
PM11	average	H09-H12	Ofloxacin	DNA topoisomerase,	46.00	16.00	0.00
PM12	average	A01-A04	Penicillin	wall, lactam	46.00	2.00	0.00

PRINTOUT OF PARTIAL PAGE OF .XLS WORKSHEET EXPORTED INTO EXCEL

WHAT'S ON EXPORTED MULTI-WORKSHEETS?

⇒ Meta Params worksheet: MicroPlates 1-8, listed from well A1 through H12, reporting all 12 parameters, with each parameter in its own column
⇒ pH Salt Params worksheet: MicroPlates 9 & 10, listed as above
⇒ Sensitivity Params worksheet: MicroPlates 11-40, listed as above
⇒ 8 x 12 format: 8 rows and 12 columns, wells: A1 – H12, representing each parameter, with MicroPlates arranged vertically and parameters arranged horizontally